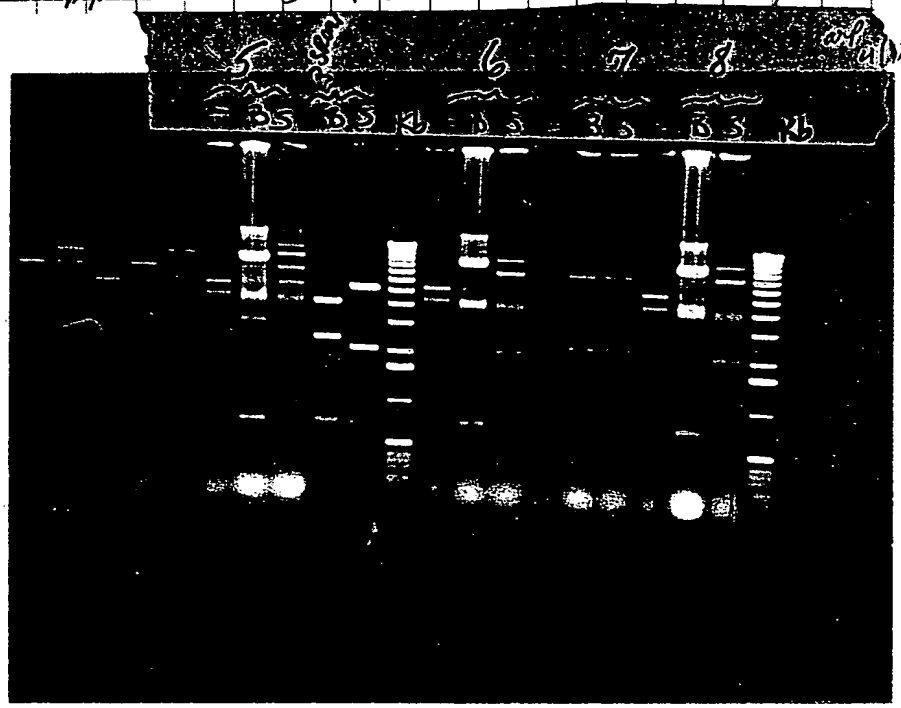
ıg N .__

Con'd from 3884 NB 2/18/95 wed

MINI PREP DNA

- cfg 500 µl of cells for 1 minute in an eppendorf cfg (centrifuge)
- removed supernatant (saved) and resuspended pellet in 100 µl of 1x PEBI (SI)
- added 200 µl of alkaline - SDS mix
- placed the tubes on ice for few minutes. (3-5 min)
- added 150 µl of 7.5 M Ammonium Acetate
- Mixed the tubes by inverting
- cfg the tubes for ~ 7-10 min.
- Transferred 400 µl supernatant to the new eppendorf tube
- added 800 µl of ethanol to supernatant. Mixed tubes.
- incubated the tubes for ~ 2 min. Spin.
- dissolved pellet in 50 µl of TE + RNAse A.
- applied 5 µl to a 1% agarose gel.



SI = 0.9% glucose
25 mM Tris HCl (pH 8.00)
10 mM EDTA

alkaline - SDS mix = 1% SDS
0.1 N NaOH

To Page No._____

ssed & Understood by me,	Date	Invent d by	Dat
		Recorded by	4/12/95

Project No._____
2 Book No._____ TITLE _____

From Page No.__

2/9/95 Thu

Purification of m13 SSDNA

1. Cfg 1.0 ml of *infected cell culture for 2 min. (1 to 5 min)
2. Transferred 800.0 µl to the new tubes (pellet was saved for isolation of RF DNA)
3. Cfg supernatant again to remove any residual cells
4. added 200.0 µl of 20% PEG + 1.5 M NaCl. Vortexed
5. Incubated tubes at room temperature for 5 min.
6. Cfg tubes for 5 min. & discarded supernatant (sup.)
7. added 200 µl of *TE & vortexed really good.
8. Cfg for ~ 1-2 min. (to remove any residual cell debris)
9. transferred sup. to the new tubes. (RNaseA can be added here)
10. added equal vol. of phenol/chloroform/isoamyl alcohol (25:24:1) Mixed well.
11. Cfg 5 min.
12. removed the aq. (upper) layer to a new tube (be very careful)
13. added 1/10 vol. of 3M NaAc. + 2½-3 vol. of 95% ↓
    { 20.0 µl NaA
    { 600.0 µl Et
14. Incubated @ -70°C till 2/14/95.

TE ($T_{10}E_1$) = 10 mM Tris-HCl pH 8.0 + 1 mM EDTA pH 8.0)

infected cell culture = ① Grew an E. coli F' strain to an OD of 0.4 in 2x YT (next pag)

F' = fertility factor: codes for tra genes & pilis to allow infection of the m13 phage. (transfer of DNA)

Cont'd ___ To Pag No

Witnessed & Understood by me,	Date	Invented by	Date
Donna Sorg	4/12/95	R c rd d by	4/12/95

Page No.

2/9/95.

Contd...

② Inoculated 1.0 ml of the cells with the phage

③ Incubated the phage infected cells at 37°C for 5 hours. Now supernatant was processed for isolation of ssDNA & cells (pellet) were ready for isolation of RF (Replicating form) dsDNA.

2/14/95 Tues.

1. Poured 0.8% Agarose gel (250 ml Volumes) in 1X TAE Buffer

2 g Agarose
250.0 ml 1X TAE Buffer

- weight the flask
- boiled for 4.20 min.
- weight the flask & adjusted Volume to before boiling with (brought up the) distilled water
- poured it on the plate.

2. 2X YT

added: 19.3 g Tryptone
12 g Yeast Extract
12 g NaCl
} brought total Volume (TV) to 1200 ml with water

- made 5 aliquots (1) 500.0 ml (2) 250 ml (3) 100.0 ml (4) 100.0 ml (5) 100.0 ml
- autoclaved at low pressure for 20 min.

3. 2X YT Top (soft) Agar

added: 0.35 g Agar
50.00 ml 2X YT
} made 3 different aliquots.

- autoclaved at low pressure for 20 min. (same as 2X YT)

To Page No.

Read & Understood by me,	Date	Invented by	Date
[signature]	4/12/95	Recorded by [signature]	4/12/95

[redacted]

From Pag No.__

4. started 2, 10 ml cultures

#1 CJ236 in 2XYT + Cm 5 ug/ml +/- phage from T.nea/m13 cloning at 37°C air-shaker
10 ml CJ236 in 2XYT
50 ul Cm 5 ug/ml 1000 ug = mg/ml = 1000 ul

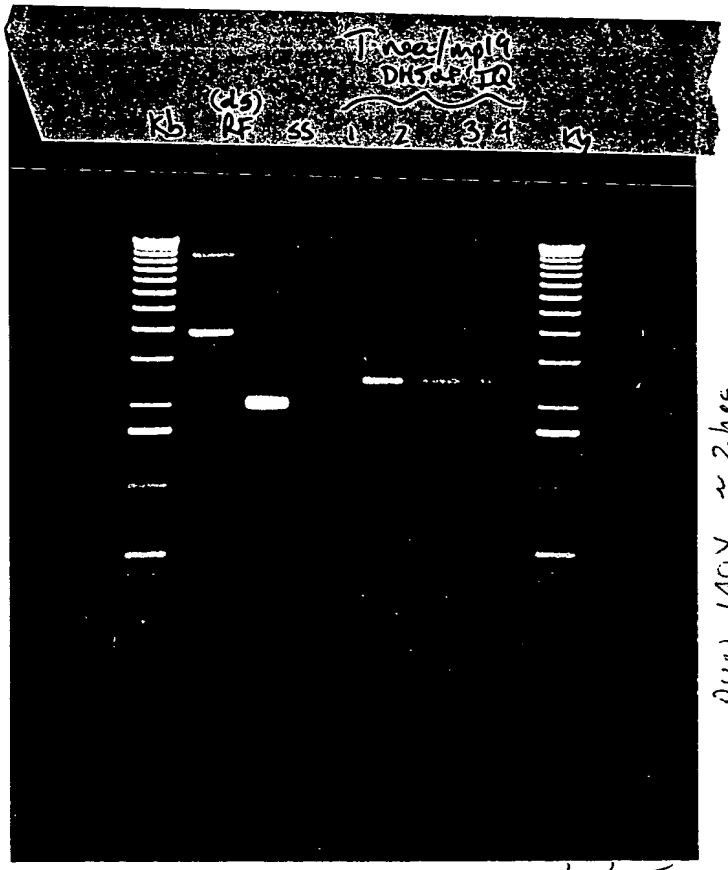
#2 10 ml Culture of T.nea/pTTC and T.nea/pTTQ 11B in LB + 100 ug/ml Ampicillin at 30°C air-shaker.
added: 10 mL
5 ul.

5. Centrifuged (cfg) ssDNA from DH5α F'IQ (tubes left at -70°C 2/9/95) for 10 min. at room temperature.
• discarded supernate
(a) Rinsed the precipitate (ppt.) with 70% EtOH. Removed any remai residual EtOH with another quick spin.
(b) Dried the DNA pellet at 55°C heat block
(c) Dissolved the DNA in 50.0 ul of TE
(d) applied 5.0 ul. on a 0.8% Agarose gel (made today).
m13 ssDNA was used as a control

T Pag N

Witnessed & Understood by me,	Date	Inv nted by	Dat
	4/12/95	R c rd d by	4/12/95

ig N .



RUN 140Y ~ 2hrs.

amp 4/12/95

To Pag N .

sed & Understood by me,	Date	Invented by	Date
[signature]	4/12/95	Record d by [signature]	4/12/95

[redacted]

Project No._____
6 Book No._____ TITLE _____

From Page No.__

2/15/95 Wed.

(+) Strand (ssDNA) lot # ED5702 260 µg/ml
RF Strand (ds DNA) lot # CC3111 5 µg/18.4 µl

calculation: ssDNA = 260 µg/ml = ng/µl

$\frac{260\ \mu g/ml}{1000\ ng/\mu g}$ 1000 ng/µg ml · 1000 µl

= 0.260 µg/µl.

1000 ng/µg (.260 µg) = 260 ng/µl

$\frac{260\ ng/\mu l}{x\ \mu l} = 100\ ng$

$260\left(\frac{1}{2.6}\right) = 100\ ng$
or
$\frac{260}{2.6} = 100\ ng.$

for 2.6 total or final volume you need 1.0 µl DNA

∴ 1 µl DNA (260 ng/µl)
1.6 µl TE
2.6 µl

for 100 ng/µl, multiply by 2 { 2.0 µl DNA (260 ng/
3.2 µl TE

ds DNA = 5 µg/18.4 µl.

1000 ng/µg × 5 µg = $\frac{1000\ ng(5\ \mu g)}{\mu g}$ = 5000 ng/18.4 µl

$\frac{5000\ ng}{18.4\ \mu l} = \frac{272\ ng/\mu l}{x\ \mu l} = 2.72\ ng.$

for 2.7 total volume you nee
1.0 µl DNA

Total Volume (TV)

T Pag N

Witnessed & Understood by me,	Date	Invent d by	Dat
	4/12/95	R c rded by	4/12/95

Pag No.

Tube # 1

	RF (ds)		
DNA	1.0 µl	x 3	= 3.0 µl
TE	1.7 µl	x 3	= 5.1 µl
TV	2.7 µl	x 3	= 8.1 µl

Tube # 2

⊕ ss DNA

DNA = 1.0 µl x 3 = 3.0 µl
TE = 1.6 µl x 3 = ~~5.4~~ 4.8 µl
TV = 2.6 µl x 3 = 7.8 µl.

Tube # 1, 2, 3, 4 of RF (ds DNA)

	① AluI	② Hind III	③ Sau3A1	④ BamHI	
H_2O	16.0 µl	→	→	→	(all 4 tubes w/ 16.0 µl
10 x Buffer	2.0 µl (React 1	React 2	React 4	React 3)	→
DNA	1.0 µl	→	→	→	

AluI	+	−	−	−
Hind III	−	+	−	−
Sau3A1	−	−	+	−
BamHI	−	−	−	+

Tube # 1, 2, 3, 4 of + (ss DNA) same order as RF

2 tubes were set-up for uncut, 1 with RF & 2nd with ⊕
- each tube added 16.0 µl H_2O
  2.0 µl React 2 10X buffer
  1.0 µl DNA
- Put all 10 tubes in
- ran the sample (all 10) on a gel next morning. (0.8% agarose gel, 147 Volts)
- picture of the gel is on the next pg (pg # 8)

To Pag No.

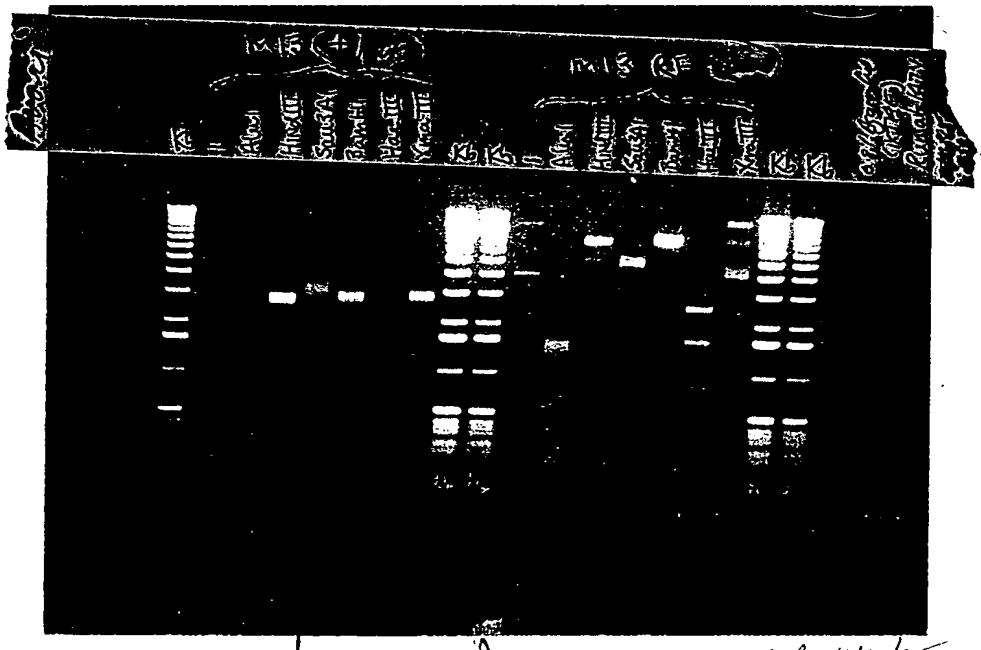
ssed & Understood by me,	Dat	Inv nted by	Date
[signature]	4/12/95	Record d by [signature]	4/12/95

[redacted]

Tube# 1 T.nea /pTTC Tube# 2 T.nea /pTTB

1.0 ml 1.0 ml

- Cfg. for 1 min. at room temperature
- discarded supernate and added: 100 µl S1 to the pellet. mixed
  200 µl S2 lysis put both tube ice.
  150 µl S2 with RNASE A
- Cfg for 5 min. at 4°C
- transferred 400 µl of supernatant to the new tubes.
- added 800.0 µl EtOH to the supernatant
- put both tubes in the fridge till tomorrow (2/16/95)



arp 4/12/95

(+) Sau3AI - gel shift (did not cut but binded)

To Pag No

Witnessed & Understo d by m ,	Dat	Inv nt d by	Date
	4/12/95	R corded by	4/12/95

Pag N

2/21/95 TUE

DIGEST T.nea/pSPORT with SstI & SphI
DIGEST M13 mp 18 & M13 mp18 w/ SstI & SphI

M13 mp 18 RF (0.44 µg/µl) } cut 500.0 ng
M13 mp 19 RF (350.0 µg/ml) }

→ 1000 ng/µg x 0.44 µg = 440 ng
500 ng / 440 ng = 1.1 µl

→ 1000 x 0.350 µg/µl = 350 ng
~~350~~ 500 ng / 350 = 1.4 µl

mp 18		mp 19	
H_2O -	35.0 µl	H_2O -	35.0 µl
10x buffer -	2.0 µl ← REact 2 →	10x buffer	2.0 µl
500ng) DNA -	1.1 µl	DNA	1.4 µl
1µl) SstI -	2.0 µl	SstI	2.0 µl
	40.0 µl		40.0 µl

T.nea / pSPORT

H_2O -	81.0 µl
10x buffer -	10.0 µl
ng/µl) DNA -	4.0 µl
SstI -	5.0 µl
	100.0 µl

- Incubated all 3 tubes @ 37°C for ½ hour
- Made 0.8% agarose gel
  250.0 ml TE buffer
  2.0 g Agarose
- boiled for 4.0 min.
- added 12.0 µl E. Bromide
- poured the gel

To Pag No.

s d & Understood by me,	Date	Invented by	Dat
[signature]	4/12/95	Recorded by [signature]	4/12/95

[redacted]

From Page N

added: 2.0 µl of 1M KCl 2 µg = 2000 ng
to 40.0 µg (mp 18 & 19)

5.0 µl of 1M KCl
to 100.0 µg (pSPORT)

added ~~SPH~~ SphI - 2.0 µl mp 18
2.0 µl mp 19
5.0 µl pSPORT

• Incubated @ 37°C for ½ hour • put the tubes in the fridge till
• ran samples on the gel ~~tomo~~ on 2/22/95

arp 2/22/95 ①

M13mp18 & mp19 RF D
are ds, supercoiled forms
the DNAs of phages M13
& 19. Using this vecto
foreign DNA can be
inserted into the mul
cloning site in an
oriented fashion.

To Pag N

Witnessed & Understood by me,	Date	Inv nt d by	Date
	4/14/95	R c rd d by	4/12/95

ag N .__ 2/22/95

1. Grow cells overnight (o/N) 10.0 mL

= 9.0 mL (1.0 mL in ea. nine tubes). Each tubes labelled DH10B ptzC/T. nea 2/22/95 BJS; LB + AP100

• Quick freeze all nine tubes in a powdered & Dry ice.

GENE CLEAN

a) Did electrophoresis on yesterday's DNA (2/21/95) M13 mp 18 and M13 mp 19 and pSPORT
b) Took the picture of the gel
c) cut off mp18 fragment, mp 19 fragment & pSPORT fragment from the gel & transformed the gel into separate eppendorf tubes.
d) added 700.0 µl NaI to each 2 tubes. Vortexed mp18 & mp19 tubes.
e) Incubated both tubes @ 55°C to melt agarose. mixed after incubation
f) added 5.0 µl Glass Milk to both tubes.
g) Incubated both tubes on ice for 5 min.
h) Cfg. both tubes (quick spin)
i) discarded supernate
j) added 500.0 µl New Wash buffer
k) discarded supernate & again added 500.0 µl New Wash buffer. washed both tubes 3 times.
l) added 10.0 µl dH_2O to the tubes. mixed well by vortexing. 55°C for 2-5 min
m) set up Ligation

Ligation

H_2O = 12.0 µl	H_2O = 12.0 µl
(ligase) 5 x Buffer = 4.0 µl	5x buffer = 4.0 µl
mp 18 DNA = 2.0 µl	mp 19 DNA = 2.0 µl
(1 u/µl) Ligase = 2.0 µl	Ligase = 2.0 µl
TV = 20.0 µl	TV 20.0 µl

n) Incubated both tubes overnight @ room temperature (cont'd)

T Pag No.____

ed & Understood by m ,	Dat	Inv nted by	Dat
	4/12/95	R cord d by	4/12/95

From Page No.

(cont'd)
T.nea/Ptrc Ep pttc

- 1.0 mL of ea.
- cfg.
- discarded supernate
- added 100.0 µl S1 . mixed well
- Incubated on ice for few min.
- added 200.0 µl S2 lysis
- Incubated on ice for few min.
- added 150.0 µl S3 w/ RNAase A
- cfg. for 7.0 min. @ 4°C

T Pag

Witnessed & Understood by me,	Dat	Invented by	Date
	4/12/95	R c rd d by	4/12/95

Project No._____
20 Book No._____ TITLE _____

From Page N .__

2/28/95 TUE

I ~~set up digest~~ DNA ppt.

① M13mp 18, ② mp 19 and ③ T. nea / pSPORT

1. - To ea 3 added 100.0 µl TE
   - " " 10.00 µl NaAc
   - " " 300.00 µl EtOH
   } to ppt. DNA
2. Incubated on dry ice for ~5 min.
3. Cfg. for 10 min. @ room temp. (no ppt.) &
4. no ppt., added 2.0 µl (carrier molecule) Yeast / tRNA. Vortexed
5. incubated on dry ice for ~5 min.
6. Cfg for 10 min. @ room temp. (Supernate saved) pellet was seen on mp 18 &
7. added 200.0 µl 70% EtOH to the pellet
8. Cfg. discarded supernate, air dried by putting tubes on the heat block.

II DIGEST set-up (to map Bam HI site)

- Cut T. nea / pSPORT with Hind III, Bam HI, Xba, NOT I, Sst, ECOR
  Separate

H_2O - 13.0 µl
buffer - 2.0 µl
T.nea/pSPORT DNA - 3.0 µl.
enzyme - 2.0 µl
TV = 20.0 µl.

enzymes - Hind III, Xba, Sst had REact 2 buffer.
- Bam HI, NOT & ECOR I had REact 3 buffer.

Control: H_2O - 13.0 µl
(REact 2) buffer - 2.0 µl
DNA - 3.0 µl

1. for separate enzymes

- Incubated @ 37°C
- ran on the gel on 3/1/95 (wed) picture shown pg 21

T Pag N

Witnessed & Understood by me,	Date	Invent d by	Dat
	4/12/95	R cord d by	4/12/95

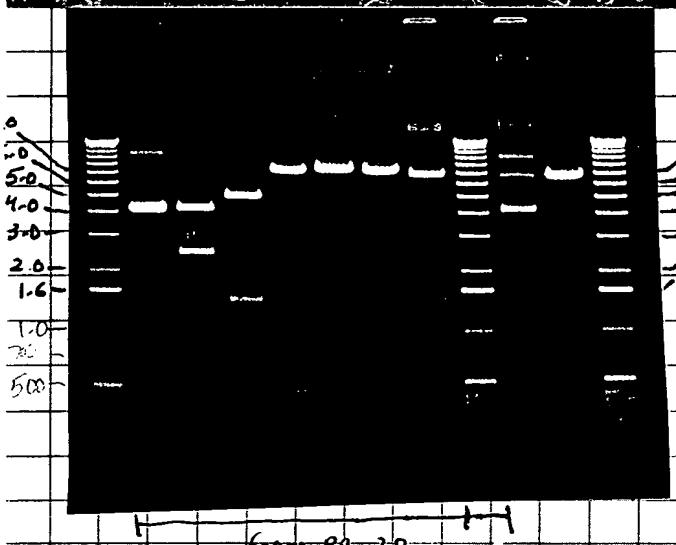
Page No.

I DIGEST set-up

- Cut ptac/T.nea w/ SstI enzyme

		Control :	
H_2O	11.0 µl	H_2O	11.0 µl
ct 2) buffer	2.0 µl.	buffer	2.0 µl.
-T.nea) DNA	2.0 µl.	DNA	2.0 µl
SstI	5.0 µl.		
TV	20.0 µl.		

- Incubate @ 37°C
- scan DNA on a gel on 3/1/95 (wed). picture shown below



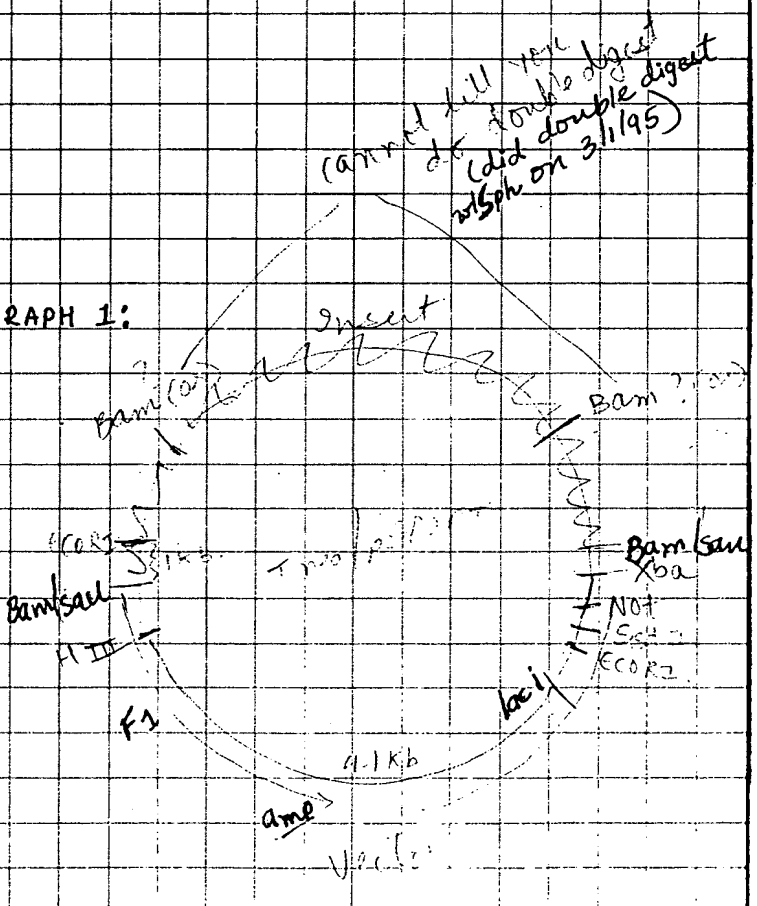
from pg 20

BamHI
10 kb (4100)
3.5 kb (2)
600 bp

EcoRI
5.0 Kb
1.5 kb
600 bp
500 bp

HindIII
4.0 Kb

: GRAPH 1:



: GRAPH 2: pg 23 of this book.

To Page No.

Signed & Understood by me,	Date	Invented by	Date
	4/12/95	Recorded by	4/12/95

Project No._____
22 Book No._____ TITLE _____

From Pag No.__

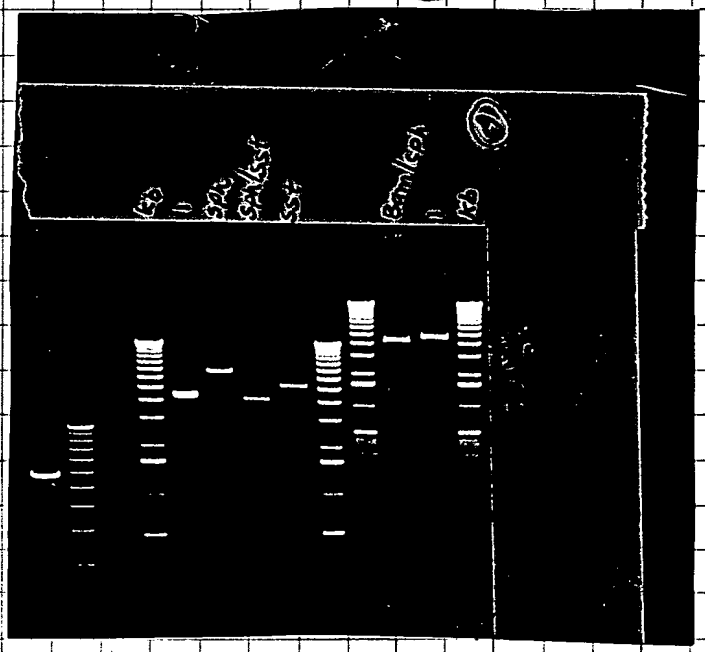
3/2/95

- ① 1 kb ladder ② T.neal/pSPORT uncut, ③ Sst, ④ Sst/Sph, ⑤ Sph, ⑥ 1 kb ladder. (from 2123 added loading dye, electrophoresed @ 190 V

- ~~digested~~ double digested BamHI/SphI (to map the Bam site T.neal

	H_2O - 14.0 µl	Control:	H_2O - 14.0 µl
(REact6)	buffer - 2.0 µl	(Uncut)	buffer - 2.0 µl
1/10 dil) (T.neal/pSport)	DNA - 2.0 µl		DNA - 2.0 µl
(Bam/Sph)	enzyme - 1.0 µl ea.		
	TV = 20.0 µl.		

Incubated @ 37°C for ~~30.0 min~~. (15 min.)

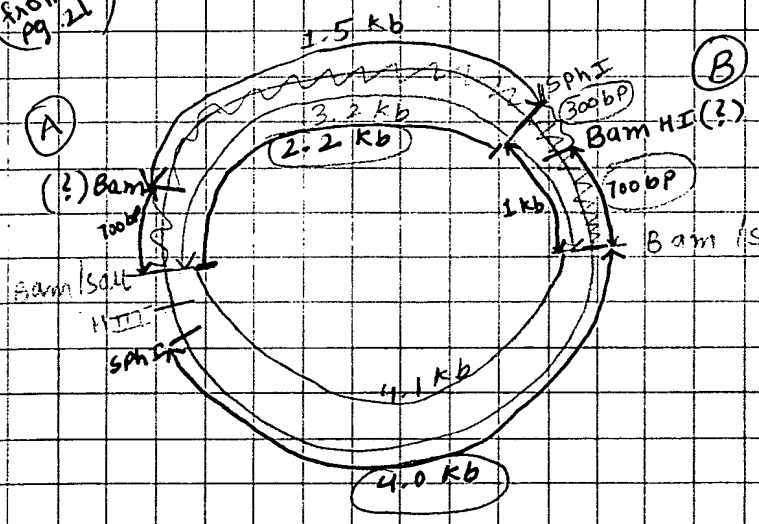


T Page 1

Witnessed & Understo d by m ,	Date	Inv nt d by	Dat
	4/12/95	R c rd d by	4/12/95

Pag N. (from pg 21)

GRAPH 2:



Bam/Sph

A
4.0 kb
700 bp
1 kb
1.5 kb

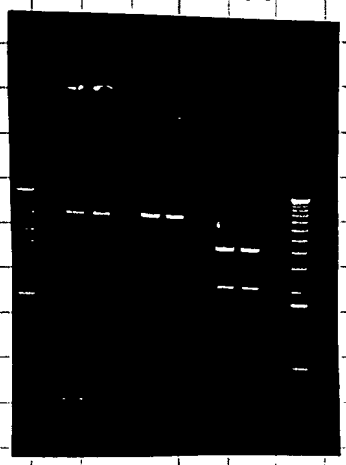
3.2
1.7
1.5

B Bam/SphI
4.0 kb
2.2 kb
300 bp
700 bp

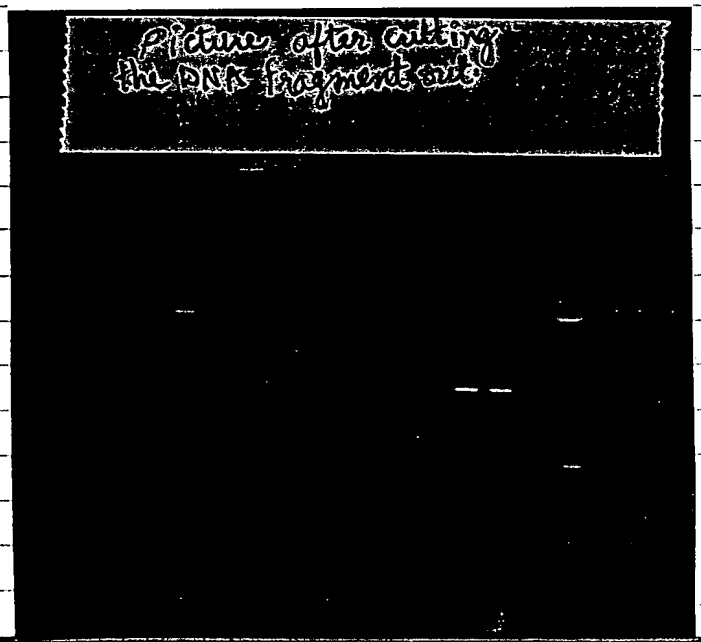
Cloning mp 18 w/ T.rea/pSPORT & mp 19 w/ T.rea/pSPORT

3/12/95

~~3/13/95~~ Thurs.



picture before cutting the ~~DNA~~ DNA fragment.



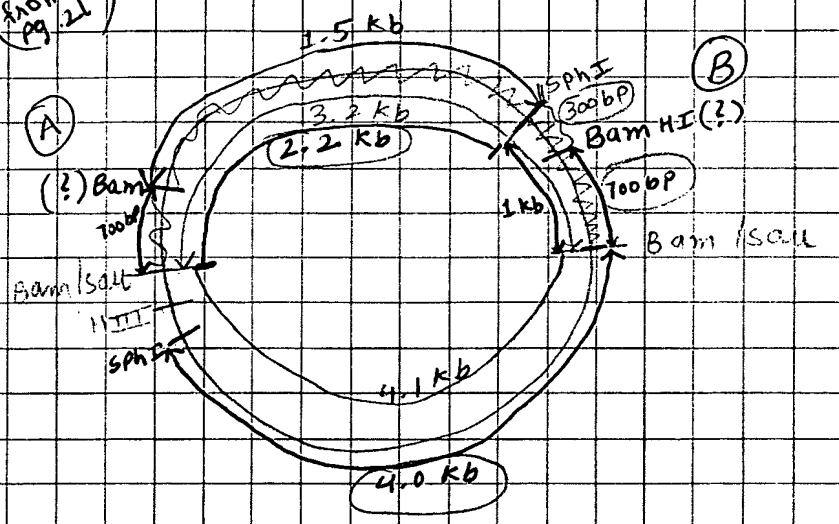
DID GENE CLEAN

To Page No._____

ssed & Understood by me,	Date	Invented by	Date
[signature]	4/14/95	Record d by [signature]	4/12/95

ag N . (from pg 21)

RAPH 2:



Bam I Sph

A
4.0 kb
700 bp
1 kb
1.5 kb

3.2
1.7
1.5

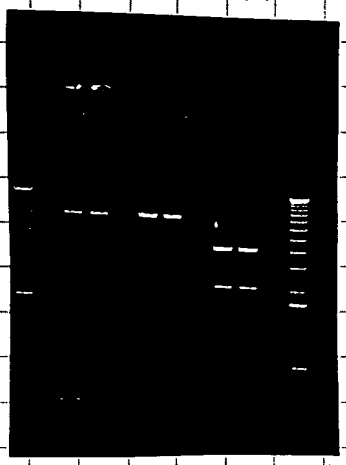
Bam I Sph I

B
4.0 kb
2.2 kb
300 bp
700 bp

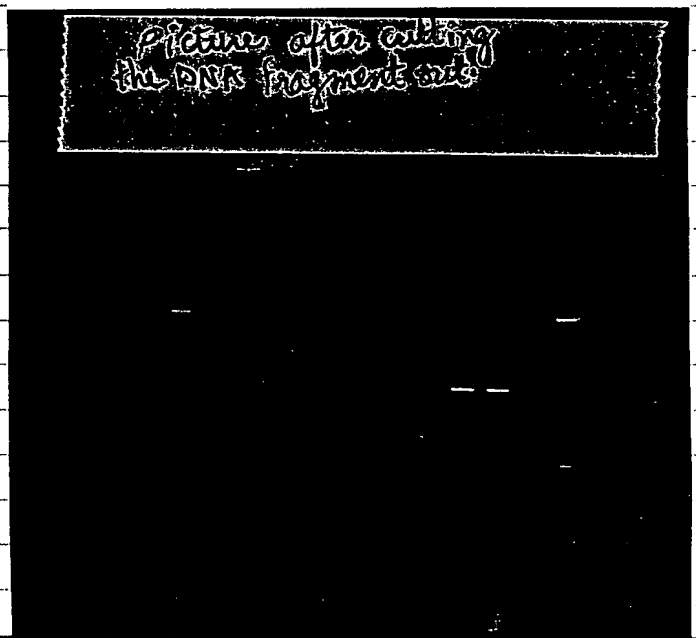
3/2/95

Cloning mp 18 w/ T.rea/pSPORT & mp 19 w/ T.rea/pSPORT

~~3/3/95~~ Thurs.



picture before cutting the ~~DNA~~ DNA fragment.



DID GENE CLEAN.

To Page No.

sed & Understood by me,	Date	Invented by	Date
	4/14/95	Recorded by	4/12/95

From Page No.

GENE CLEAN

- Mixed (cut) mp18 with T.nea pSPORT cut w/ Sst I/Sph I } 1 tube
- " mp19 with " " " " " } 2 tube

- added 700.0 µl NaI to each 2 tubes. Vortexed
- put the tubes in 55°C heat block to melt agarose
- after agarose melted, added 5.0 µl glass milk to both tubes
- Incubated both tubes on ice for 5.0 min.
- cfg. both tubes (quick spin)
- discarded supernate & washed pellet 3 x with New Wash b
- added 14.0 µl dH_2O to each tube
- quick spinned, discarded pellet & saved supernate

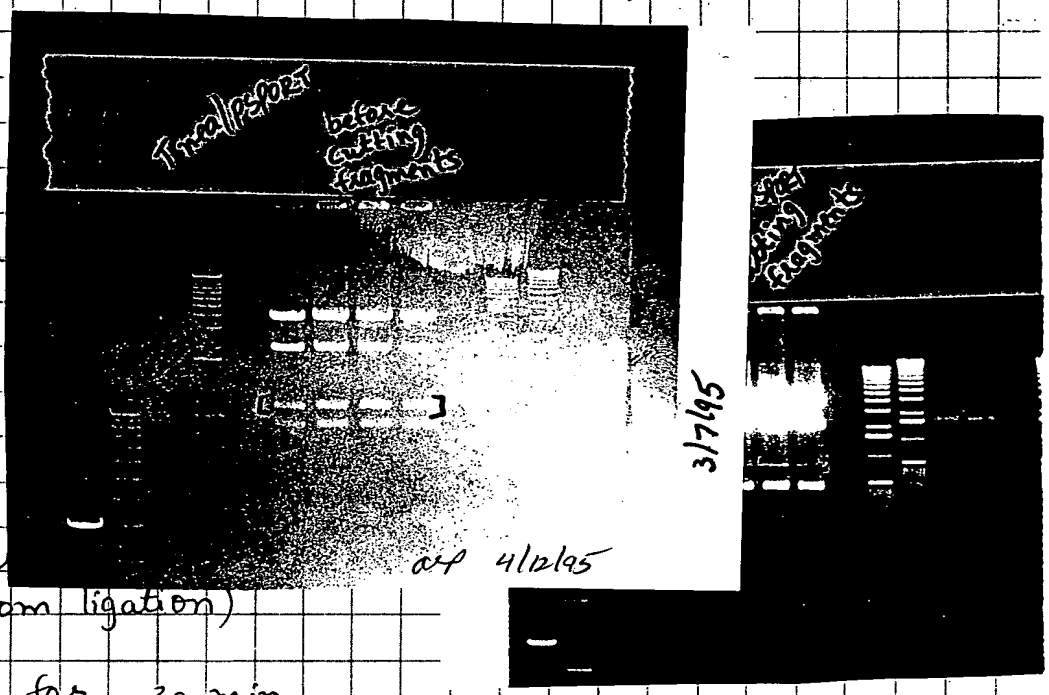
Set-up Ligation

(mp18)(mp19) DNA	- 14.0 µl
(ligase) 5x buffer	- 4.0 µl
ligation	- 2.0 µl.
TV	- 20.0 µl.

- Incubated both

Xfection Cells

(1) 100.0 µl competen
→ 3.0 µl DNA (from ligation)



(2) incubated on ice for 30 min
(3) heat shocked @ 42°C H_2O bath for 35 sec.

- melted 0.7% 2x YT top agar, added 4.0 ml to 6 different gla tubes & put the tubes @ 55°C heat block

Didn't Work

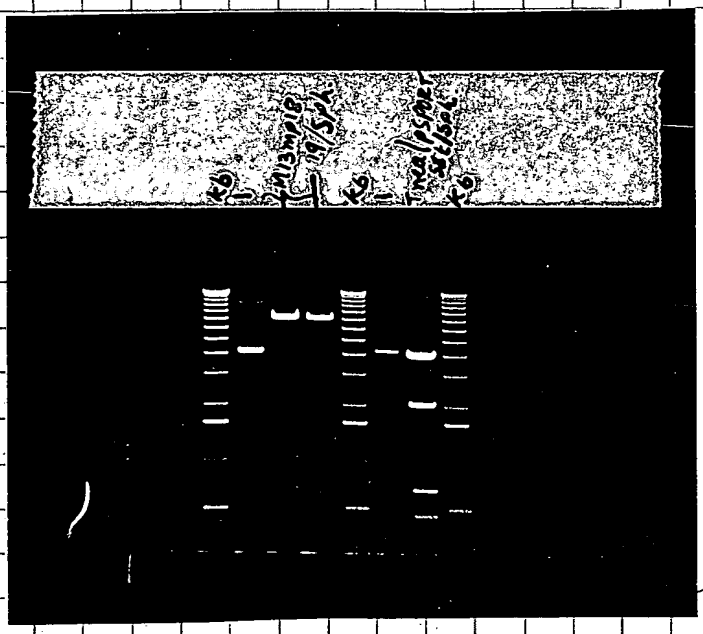
T Page N

Witnessed & Understood by m ,	Dat	Invented by	Dat
	4/12/95	Rec rded by	4/12/95

ag N

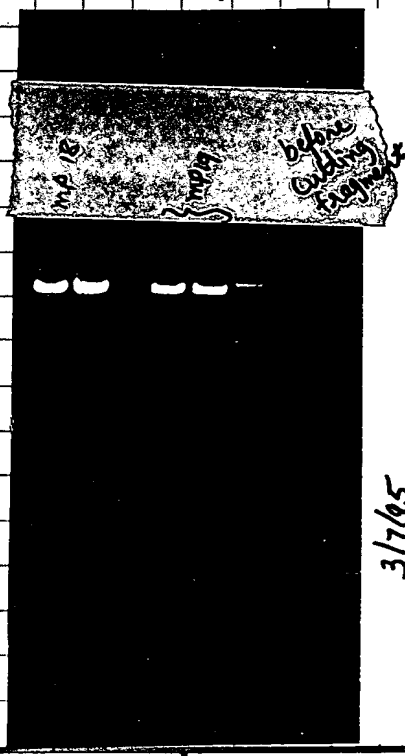
Repeat

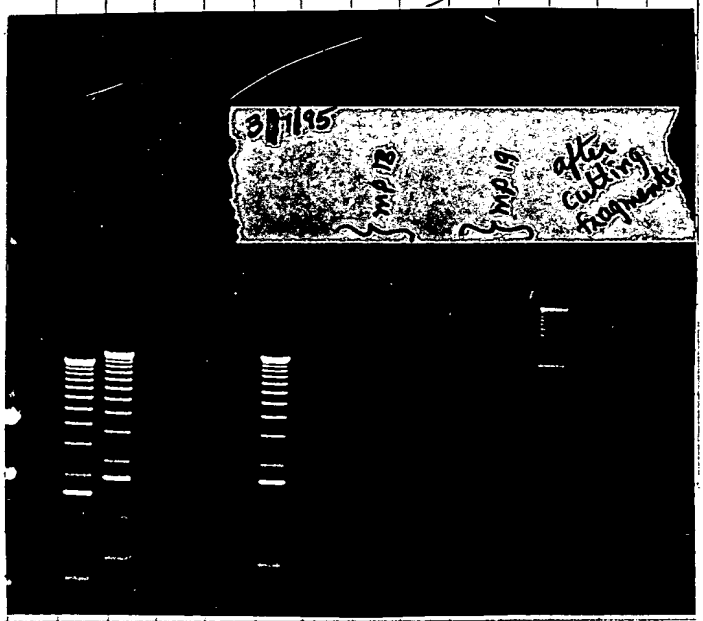
3/7/95 TUE



3/7/95

After taking picture or looking @ the gel, ~~M~~ * M13 mp 18 and M13 mp 19 is @ the 7.2 kb ~~cut~~ which was cut with SphI. ~~We decided to cut~~ We planned on cutting mp 18 and mp 19 with SstI. The gel picture below shows mp 18 & mp 19 before & after cutting the DNA fragments. After cutting the fragments performed Gene CLEAN





3/7/95

To Page No.

sed & Understood by m ,	Date	Invented by	Date
	4/12/95	Recorded by	4/12/95

[redacted]

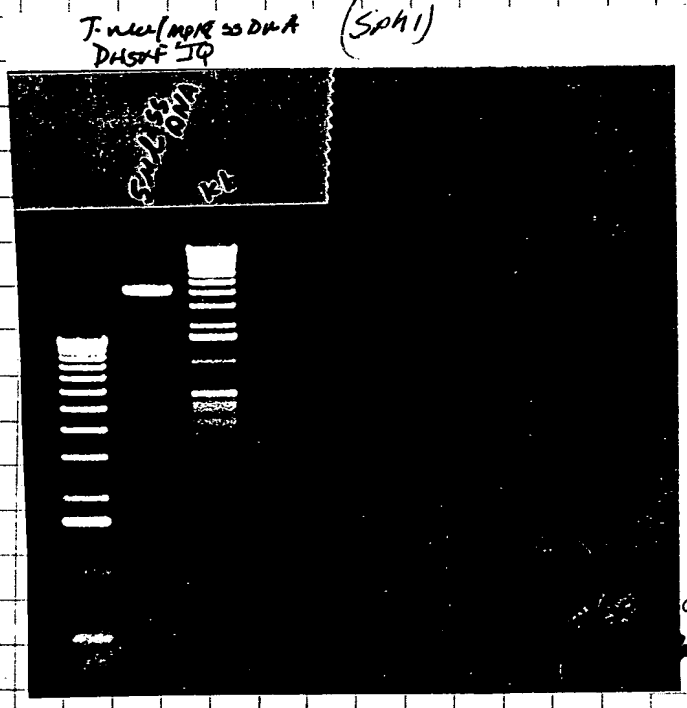
Fr m Page N .__

labelled 2 tubes, 1 w/ mp 18, & 2nd w/ mp 19

1. To the DNA w/ agarose gel, added 700.0 µl NaI
2. put the tubes @ 52°C heat block to melt agarose. vortexed (constan
3. added 5.0 µl glass milk to both tubes · mixed
4. Incubated on ice for 5 min.
5. Cfg. (quick spin) @ room temp.
6. discarded supernate, added 500.0 µl New wash buffer
7. discarded supernate, washed pellet 3x with New wash buffer
8. after washing 3x, added 14.0 µl dH_2O to the pellet (Mixed) (discarded s
9. incubated @ 52°C for 5 min.
10. discarded pellet & saved supernate for ligation.
    (cont'd this on 3/8/95 wed.)

SIGNE CLARK

Purification of m13 ss DNA (T.rea 2Kb [SphI] /mp19) from pg. 1



arf 4/12/95

To Page N

Witnessed & Understo d by me,	Dat	Inv nt d by	Date
[signature]	4/12/95	R c rd d by [signature]	4/12/95

Cell growth & Infection

- Grew an E. coli F' strain to an OD of 0.2-0.4 in 2x YT
- Inoculated 1-2 ml of the cells w/ the phage (added 10.0 µl from a liquid phage stock & added to cells)
- Incubated the phage infected cells @ 37°C for 5-7 hours.
- The supernate can now be processed for isolation of ssDNA & the cells can be processed for the isolation of Replication Form (RF) ds DNA.

Purification of m13 ssDNA

- transferred 1.0 ml culture of infected cell to 4 different eppendorf tubes
- cfg 4 tubes for 2 min.
- transfered supernate to the new tubes & saved pellet from 1 tube (out of 4 tubes) for isolation of RF DNA
- spinned the supernate again & transfered the supernate to the new tubes (done to remove any residual cells remained behind)
- passed the supernate through a 0.45 µ filter as to remaining cells (done when performing site-directed mutagenesis)
- added 200.0 µl of 20% PEG + 1.5 M NaCl. Vortexed
- Incubated tubes for 15 min @ room temperature (or overnight @ 4°C)
- cfg. for 10 min in a µ cfg. @ room temp.
- discarded supernate & briefly spinned the tubes to remove the residual soln from the side of the tube (removed as much supernate as possible)
- added 200.0 µl TE. Vortexed
- cfg for 2 min. to remove any residual cell debris.
- Transferred supernate to the new tube. (added 5.0 µl RNase I to remove any residual nucleic acid from the prep. Benzonase will remove both RNA & DNA very efficiently.
- added equal volume of phenol/chloroform/isoamyl alcohol. mixed well.
- Cfg for 5.0 min.
- transferred the upper layer to a new tube (BE CAREFUL NOT TO DISTURB WHITE INTERFACE OR REMOVE ANY PHENOL)
- added 20.0 µl NaAc & 600.0 µl EtOH
- Incubated @ -70°C for 5-15 min. (we left @ -70°C overnight)

To Page No.

ssed & Und rst od by me,	Date	Invent d by	Date
[signature]	4/12/95	Recorded by [signature]	4/12/95

[redacted]

Project No._____
28 Book No._____ TITLE _____

From Page No.__

3/8/95 wed

- cfg the samples for 10-15 min.
- discarded the supernate & rinsed the pellet w/ 70% EtOH
- Dried the pellet @ 55°C heat block or @ room temperature
- dissolved the DNA in 50.0 µl TE.

SSM Rxn

3/8/95 wed.

Annealing Rxn.

	+ Primer (-2899)	- Primer (-2899)
H_2O -	3.0 µl	4.0 µl
5x Buffer	2.0 µl	2.0 µl
89.1% SS DNA	4.0 µl	4.0 µl
(200ng/µl Kinased) Oligo	1.0 µl	—
TV	10.0 µl	10.0 µl

Incubated @ 70°C - 75°C for 2 min. (to eliminate non-spf. bi
" @ 37°C - 40°C for 2 min.

Synthesis Rxn

Annealing Rxn -	10.0 µl.
5mC 10x buffer -	2.0 µl
H_2O -	6.0 µl
T_4/T_7 DNA poly -	1.0 µl
T_4 DNA ligation -	1.0 µl
TV -	20.0 µl.

Incubated @ 37°C for 10 min.

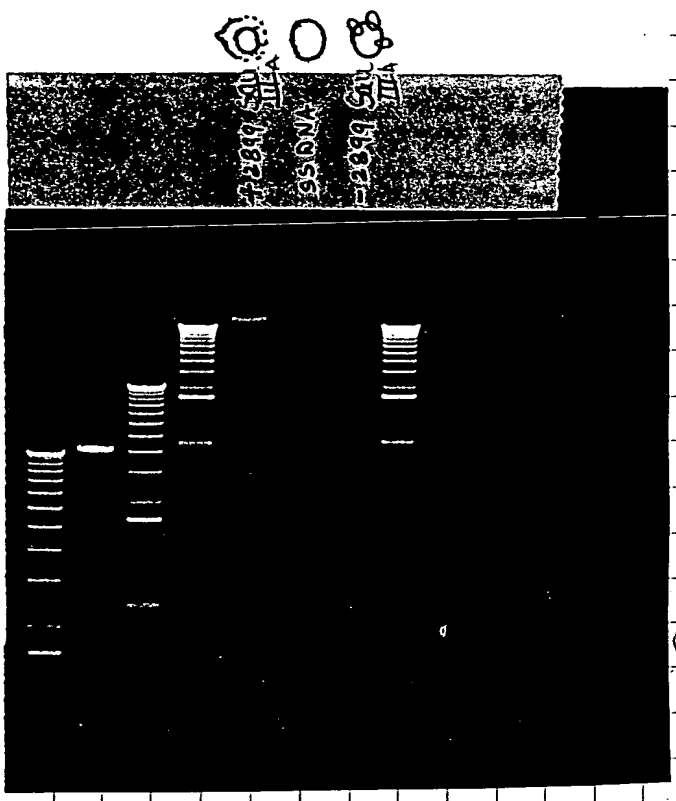
Synthesis rxn -	2.5 µl
TE -	8.0 µl
loading dye -	1.0 µl

- ran the sample on the gel
- the picture on the next page, # 29.

T Pag No

Witnessed & Understood by m,	Date	Invented by	Dat
[signature]	4/12/95	Rec rd d by [signature]	4/12/95

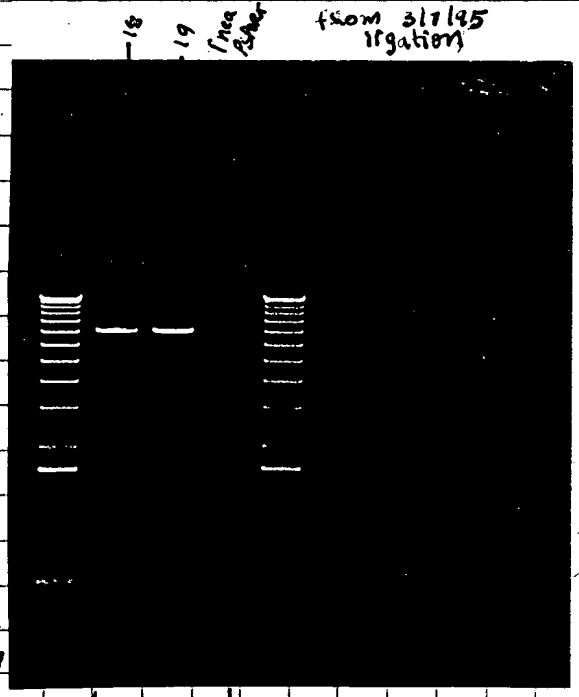
Pag N.



(con'd from pg. 28)

+2899 (universal primer) oligo forms a ds DNA ∴ +2899 fragment looks brighter because Et. Bromide binds to it better. -2899 Primer binds but it (DNA) does not hold strongly ∴ the DNA fragment looks fainted or light, less Et. Bromide is able to bind.

(con'd on pg. 41)



Ligation from 3/7/95 (pg. 26)

H_2O	-	8.0 µl	H_2O	-	8.0 µl
5X buffer	-	4.0 µl	5X buffer	-	4.0 µl
mp 18	-	2.0 µl	(vector) mp 19	-	2.0 µl
insert	-	4.0 µl	insert	-	4.0 µl
ligation	-	2.0 µl	ligation	-	2.0 µl
TV	-	20.0 µl	TV	-	20.0 µl

- Incubated both samples for 1 hour @ room temp.

- 100.0 µl competent cells
  3.0 µl DNA
  } Xfection cells.

Xfection
10% mp18 / mp19
90% mp18 / mp19
Control

Ran mp18 on 3/10 (used DNA from 3/10/95 again on 3/15/95 pg. 32)

To Page No.

sed & Understood by me,	Date	Inv nted by	Date
	4/12/95	Recorded by	4/12/95

From Page No.

— 10% mp 18 / mp 19

added → 4.0 mL 2x YT TOP Agar
100.0 µl X-Gal 4%
5.0 µl IPTG 200 mM (inducer = repressor gives tighter affinity)
60.0 µl lawn cells
10.0 µl x fection cells (after heat shock for 35 sec.)

— 90% mp 18 & mp 19.

Same way as 10%

— Control
100.0 µl X-Gal
5.0 µl IPTG
60.0 µl lawn cells.

Witn ss d & Und rsto d by me, Dat 4/12/95 Inv nt d by Dat

R c rd d by 4/12/95

Page No.__

3/14/95 TUE

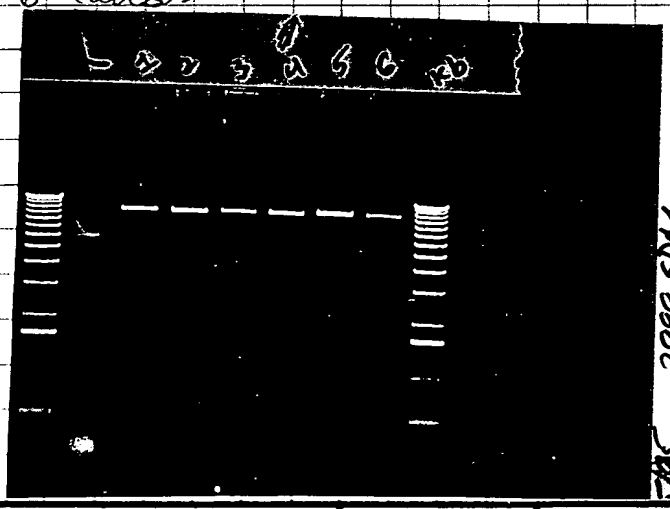
Rebecca: Miniprep

1.0 ml culture of T.rea/mp 19 grown for 5 hours @ 37°C in 6 different glass tubes
transfered 1 ml cell to the 6 different labelled eppendorf tubes.
cfg all 6 tubes for 2 min. @ room temp.
removed supernate & saved in different tubes
added 100 µl S1 mixed well
added 200 µl S2. put the tubes on ice (mixed by inverting)
added 150 µl 7.5 M NH_4OAc
incubated on ice for 5 min.
cfg for 7 min. @ room temp. (4°C) NOTE: cfg in 4°C room was taken away for repair : used @ RT
transfered supernate (400.0 µl) to the new 6 labelled tubes
added 800 µl of EtOH to the 400 µl of supernate (mixed well)
incubated @ -70°C for 30 min.
cfg for 2 min. @ room temp (discarded supernate)
rinsed w/ 70% EtOH (removed supernate)
added 50.0 µl TE to the pellet.

H_2O - 7.0 µl	x 6 tubes	=	42.0 µl
buffer - 2.0 µl	x 6	=	12.0 µl
Eco47III - 1.0 µl	x 6	=	6.0 µl
	TV		60.0 µl

added 10.0 µl DNA to each 6 tubes.

the map is on next page # 32. fragments from all 6 tubes are ~~in~~ still present, they haven't gone into the mutant.
tried miniprep again next day. (Started)

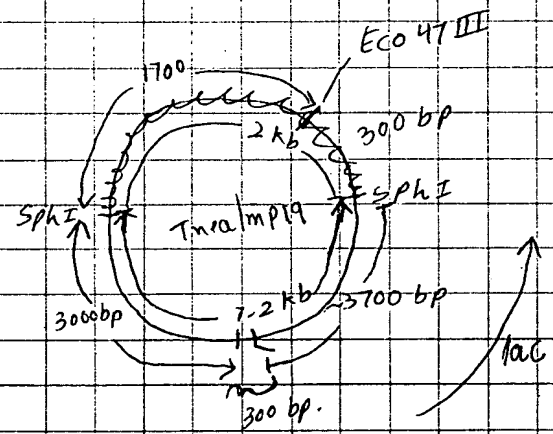


Read & Understood by m ,	Date	Invent d by	Date
Rolanp	4/12/95	R corded by	4/12/95

Project No._____
32 Book No._____ TITLE _____

From Page No.__

3/15/95 Wed



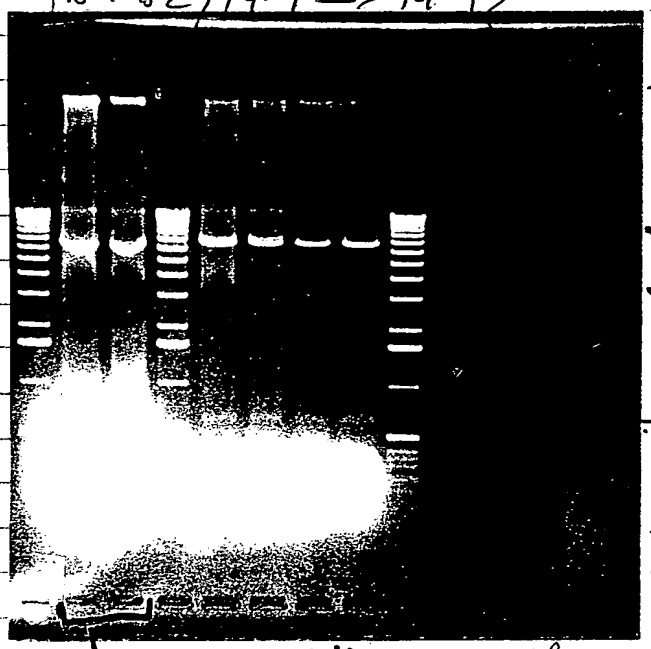
Eco 47 III

parent ~8-9 kb
0.3 kb → (most probably won't see fragment because too small & too light)

mutant 4 kb
4.7 kb
0.3 kb

DNA from date 3/10/95

(8-1 8-2) 19-1 → 19-4



3/10/95 1 hr/37°C

H_2O	= 6.0 µl.	H_2O	= 6.0 µl.
React 6 buffer.	= 2.0 µl.	buffer	= 2.0 µl
mp18 ① DNA	= 10.0 µl	mp18 ② DNA	= 10.0 µl
Sst I/Sph	1.0 µl ea.	Sst I/Sph	= 1.0 µl ea.
TV	20.0 µl	TV	20.0 µl

from pg 29 & ran again on 3/15

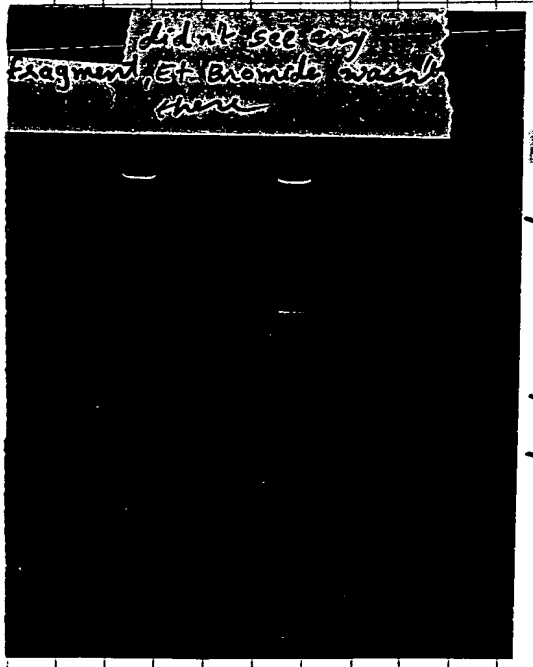
arp 4/12/95

T Pag

Witnessed & Understood by me,	Date	Invented by	Date
	4/12/95	Recorded by	4/12/95

ag No.

- Incubated both tubes @ 37°C for 30 min.
- added 2.0 µl loading dye to ea. tube
- ran both on a gel
- took picture



1.0 ml T.nea (sph I) /mp 19 +2899 + Sau 3A I grown for 5 hours @ 37°C in 10 different glass tubes
after 5 hours transferred 1.0 ml culture to the 10 labelled eppendorf tubes
cfg all 10 eppendorf tubes @ room temperature for 2 min.
removed supernate & saved
put all 10 tubes w/ pellet & all 10 tubes w/ supernate @ -70°C overnight or until 3/16/95 Thursday.

NOTE: Brian had to leave @ 4:30 pm & this was a point to stop @.

To Page No.

ssed & Understood by me,	Dat	Inv nted by	Date
	4/12/95	Recorded by	4/12/95

Project No.
34 Book No. TITLE

From Page No.

3/16/95 Thurs.

con'd from page 33 3/15/95 wed. MINIPREP

- took the pellet out from -70°C (10 eppendorf tubes)
- added 100 µl S1 mixed well
- added 200 µl S2 put all 10 tubes on ice mixed
- added 150 µl 7.5 M NH_4OAc
- incubated on ice for 5 min.
- cfg all 10 tubes for 5 min. @ room temp. (4°C)
- transferred 400 µl of supernate to the new 10 labelled tubes
- added 800 µl EtoH Mixed well
- incubated all 10 tubes for 30 min. @ -70°C.
- cfg ~~& discard~~ for 2 min. @ room temp.
- discarded supernate & washed pellet with 70% EtoH.
- added 50 µl TE to all 10 tubes w/ pellet

		tubes	
H_2O	7.0 µl	x 10	= 70.0 µl
buffer	2.0 µl	x 10	= 20.0 µl
Eco47III	1.0 µl	x 10	= 10.0 µl
	TV		= 100.0 µl

- added 10.0 µl from TV to all other 9 tubes
- added 10.0 µl DNA to each 10 tubes

- incubated @ 37°C for 30 min.
- added 2 µl loading dye
- ran all 10 samples on a gel for 1 hour @ 190 V
- took a picture

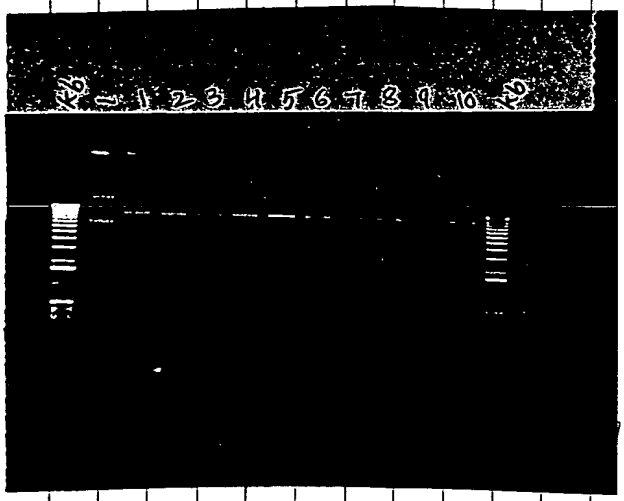
picture on pg 35

To Pag No

Witnessed & Understood by me, | Date 4/12/95 | Invented by | Recorded by | Dat 4/12/95

ag N .__

parent & mutant should look like
ECO 47 III



Eco 47 III 3/16/95 4/12/95

Parent
8.9 kb
0.3 kb

mutant
4.5 kb
4.4 kb
0.3 kb.

may probably be too light to see

NOTE: In this ~~#~~ we could see parent & some mutant. mutant is seen on # 5,6,7,8

PD

To Page No._____

sed & Und rstood by me,

Date 4/12/95

Invented by

Recorded by

Date 4/12/95